

The Differences Value in Neutrophil to Lymphocyte Ratio in Severe Preeclampsia Patients before and After Termination

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Abstract: *Introduction: Severe preeclampsia is characterized by blood pressure of more than or equal to 140/90mm Hg on two examinations within 4 hours in pregnancy over 20 weeks accompanied by proteinuria and new onset of thrombocytopenia, renal insufficiency, impaired liver function, pulmonary edema, impaired liver function. Visuals and brain. Methods: This was an observational study with a prospective cohort data collection method to assess the levels of NLR before and 48 hours after termination in preeclampsia patients treated at H. Adam Malik General Hospital, Medan. Results: This study was followed by 30 pregnant women with severe preeclampsia who had terminated their pregnancy and was treated at H. Adam Malik Hospital and Madani Hospital Medan. The mean age of the subjects was 31.73 years old, the mean age of the mother during pregnancy was 36.8 years old, the mean systolic blood pressure was 164.67 mmHg and the average diastolic blood pressure was 103.33 mmHg. The mean NLR before the termination of pregnancy was 3, 50 (SD = 2.55) and after the termination of pregnancy, the mean decreased to 3, 10 (SD = 1.90). T-dependent test analysis showed no significant difference in the mean NLR before and after the termination of pregnancy (p = 0.091). Conclusion: There is no significant difference in the mean NLR between before and after the termination of pregnancy. Keywords: Neutrophils, Lymphocytes, NLR, Severe preeclampsia, Eclampsia.*

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1. Introduction

Preeclampsia is characterized by blood pressure higher than or equal to 140/90 mmHg on two examinations within 4 hours in pregnancy over 20 weeks accompanied by proteinuria and new onset of thrombocytopenia, renal insufficiency, impaired liver function, pulmonary edema, and visual and brain disturbances. Around 2 - 8% of all pregnancies in the world have preeclampsia and are responsible for global maternal deaths as much as 12%. In Indonesia, the incidence of death due to preeclampsia is estimated to be around 7 - 10% of all pregnancies.¹

Recently, the use of neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) obtained from routine complete blood counts has been widely used as a marker of the systemic inflammatory response (SIRS), especially preeclampsia. In obstetrics, it has been reported that NLR values are increased in patients with hyperemesis gravidarum, gestational diabetes, preeclampsia, intrahepatic cholestasis in pregnancy, HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count), ectopic pregnancy, preterm labor, and other diseases. Pathogenically, the increase in neutrophil activity and lymphocyte ratio in preeclampsia is not only due to the increase in blood pressure which releases antiangiogenic factors where the increase does not occur in normal pregnancy, but it is also caused by dysregulated immune factors and endothelial damage factors. Neutrophils play a role in increasing NLR which is also a marker of SIRS.^{2,3}

Increased NLR is associated with an increased risk of cardiovascular events, diabetes mellitus, and mortality in some malignancies. NLR has also previously been used as a

predictor of complications in pregnancy but the results have been inconsistent. For example, the NLR was not found to be satisfactory for predicting hypertension in pregnancy.⁴ However, in another similar study, it was found that the NLR was increased in patients with preeclampsia.² However, further research is needed on the predictive value of the incidence of preeclampsia from the use of NLR inflammatory markers.⁵ Research by Yavuzcan et al (2014) found that NLR was higher in patients with severe preeclampsia than in non - pregnant women (p=0.000), but there was no difference in NLR values in patients with severe preeclampsia compared to normal pregnancies (p=0.721). The lymphocyte count is higher in patients with severe preeclampsia than in normal pregnancies.⁶

Interpretation of laboratory values such as increased white blood count is interesting to know further. Pregnancy involves a wide variety of physiological changes, resulting in a special preference value for laboratory testing. Based on the above background, the researcher wanted to know the difference in NLR levels in the preeclampsia patient group before termination compared to the preeclampsia patient who had been terminated and also to test how strong the NLR variable was.

2. Method

This research was an observational study with a prospective cohort review of data to assess the level of NLR before and after termination in preeclampsia patients treated at H. Adam Malik General Hospital, Medan. Ethical clearance from the Health Research Committee, Faculty of Medicine, University of North Sumatera, and research permission from the Research and Development Installation of Haji Adam

Malik Hospital, Medan. Informed consent was requested in writing from the research subjects or represented by their families.

The research was conducted at the Department of Clinical Pathology, University of North Sumatera Medical Faculty / H. Adam Malik Hospital, Medan in collaboration with the Department of Obstetrics and Gynecology, University of North Sumatera Medical Faculty / H. Adam Malik Hospital, Medan, from May 2022 - July 2022. The population of this study was pregnant patients with preeclampsia. who were treated at H. Adam Malik Hospital Medan from May 2022 - July 2022. The subjects of the study were pregnant patients with preeclampsia who were treated at H. Adam Malik Hospital Medan and had met the inclusion criteria. The collection of research subjects was carried out consecutively and stopped when the number of samples had been reached. The inclusion criteria were pregnant women with severe preeclampsia who were willing to participate in the study. Exclusion criteria were pregnant women with chronic diseases (hypertension, diabetes, chronic kidney disease), and pregnant women with severe or autoimmune infections. Dropout criteria are patients who died.

Preeclampsia is diagnosed with an increase in blood pressure of up to 140 mmHg systolic or 90 mmHg in diastolic pressure and the presence of protein in the urine of 300 mg or more in a 24 - hour urine specimen or 300 mg/dL in a random urine specimen that occurs after 20 weeks. gestational age, assessed using a urine dipstick. After that, the patient was examined for NLR before and 48 hours after termination of pregnancy, by taking 5 cc of blood from the median cubital vein, then the blood in the EDTA tube was immediately homogenized. NLR examination was carried out using an automatic cell counter analyzer Sysmex XN - 1000 with a flow cytometry method. The results of a complete blood count in the form of NLR values will be entered into the data of each research sample based on their respective medical records using the Laboratory Information System (LIS).

Data analysis was performed using SPSS (Statistical Package for Social Sciences, Chicago, IL, USA) software for Windows. The description of the characteristics of the research subjects is presented in tabulated form and described. The difference between NLR levels in preeclampsia patients using a dependent T - test (paired T - test) if the data are normally distributed. If the data is not normally distributed, the Wilcoxon test is used. All statistical tests with p - value < 0.05 were considered significant.

3. Results

This study was followed by 30 pregnant women with preeclampsia who had terminated their pregnancy and were treated at H. Adam Malik General Hospital Medan. All subjects involved in this study met the inclusion criteria. The complete characteristics of the subjects are shown in table 1.

Table 1: Characteristics of research subjects

Characteristics of subjects	n = 30
Age, years old	
Mean (SD)	31.73 (6.29)
Age of marriage, years old	
Mean (SD)	26.5 (4.07)
Age when pregnant, years old	
Mean (SD)	36.8 (1.27)
Body weight, kg	
Mean (SD)	68.6 (8.62)
Height, cm	
Mean (SD)	156.67 (4.84)
Systolic blood pressure, mmHg	
Mean (SD)	164.67 (16.97)
Diastolic blood pressure, mmHg	
Mean (SD)	103.33 (7.58)
Ethnic, n (%)	
Batakese	4 (13.3)
Javanese	5 (16.7)
Karonese	2 (6.7)
Mandailing	17 (56.7)
Minang	2 (6.7)
Antenatal Care History, n (%)	
At hospital	3 (10)
Public health centre	3 (30)
Midwives	24 (80)
Family history, n (%)	
Hypertensive father	3 (10)
Hypertensive mother	26 (86.7)
None	1 (3.3)
Labor delivery, n (%)	
Sectio caesarea	30 (100)
Proteinuria, n (%)	
Negative	1 (3.3)
+2	2 (6.7)
+3	21 (70)
+4	6 (20)

Table 2 shows the characteristics of neonates based on the assessment of the APGAR score and birth weight.

Table 2: Characteristics of neonates

Characteristics	n = 30
APGAR score, n (%)	
0/0	2 (6.7)
4/5	1 (3.3)
6/7	2 (6.7)
7/8	21 (70)
8/9	4 (13.3)
Birth weight, gram	
Mean (SD)	2920 (328.42)
Median (Min - Max)	2900 (2500 - 4000)

Based on the assessment of the APGAR score, most of the 21 neonates (70%) showed an APGAR Score of 7/8. The average birth weight was 2920 grams.

Table 3 shows the levels of neutrophils, lymphocytes, and the ratio of neutrophils to lymphocytes before and after the termination of pregnancy.

Table 3: Neutrophil, lymphocytes, and neutrophil to lymphocyte ratio (NLR) before and after the termination of pregnancy

	Before termination	After termination	p
Neutrophil			
Mean (SD)	78.50 (2.22)	68.70 (2.21)	<0.001 ^a
Median (Min - Max)	74.60 (66.50 – 82.70)	70.35 (65.40 – 75.30)	
Lymphocyte			
Mean (SD)	22.40 (0.47)	22.10 (0.39)	0.010 ^a
Median (Min - Max)	22.25 (21.90 – 22.60)	22.00 (21.80 – 22.20)	
NLR			
Mean (SD)	3.50 (2.55)	3.10 (1.90)	0.091 ^b
Median (Min - Max)	5.38 (1.07 – 9.70)	4.29 (1.08 – 7.50)	

The mean NLR before the termination of pregnancy was 78.50 (SD = 2.22) and after the termination of pregnancy, the mean decreased to 68.70 (SD = 2.21). The results of the analysis using the dependent T - test showed that there was no significant difference in the mean NLR before and after the termination of pregnancy (p < 0.001).

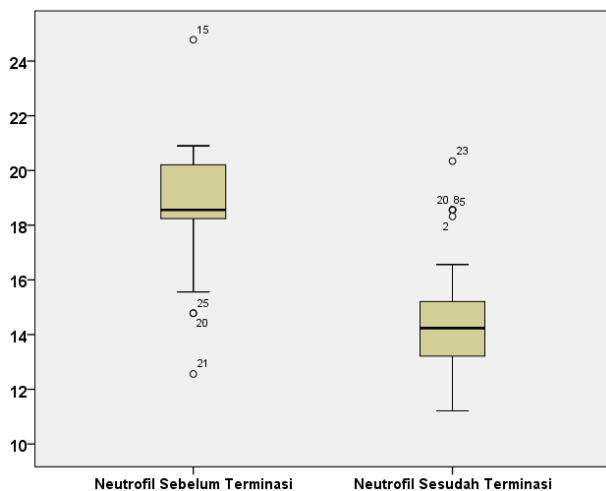


Figure 1: Boxplot graph of neutrophil levels before and after termination of pregnancy

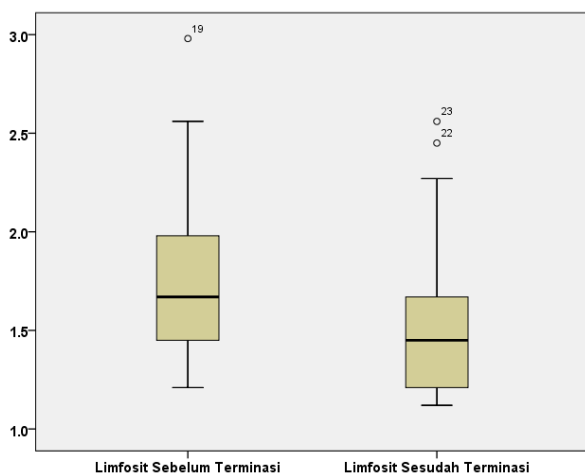


Figure 2: Boxplot graph of lymphocyte levels before and after termination of pregnancy

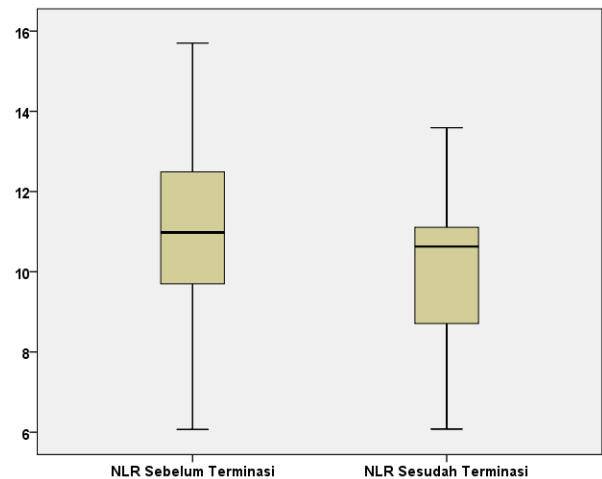


Figure 3: Boxplot graph of neutrophil to lymphocyte ratio (NLR) before and after the termination of pregnancy

4. Discussion

This study included 30 pregnant women with preeclampsia who had terminated their pregnancy and were treated at Haji Adam Malik General Hospital Medan. The mean age of the subjects was 31.73 years with the youngest age being 23 years old and the oldest being 45 years old. The average weight and height were 68.6 kg and 156.67 cm, respectively. Based on gestational age, it shows an average of 36.8 years with the youngest pregnant age being 34 years and the oldest pregnant at 39 years. The mean systolic and diastolic blood pressure are 164.67 mmHg and 103.33 mmHg, respectively.

Age under 20 years and above 35 years are also called the age at high risk for complications of preeclampsia in pregnancy. At the age of under 20 years, the uterus has not reached the normal size for pregnancy, so the possibility of disruption in pregnancy is greater. At the age of over 35 years, a degenerative process occurs which results in structural and functional changes in the peripheral blood vessels, making them more susceptible to preeclampsia. In addition, the condition of the reproductive organs at the age of over 35 years has undergone endothelial changes that are no longer ready to accept the pregnancy, and have a greater risk of experiencing a tendency to increase blood pressure, thereby increasing the occurrence of preeclampsia.⁷

Based on gestational age, most of the study subjects with preeclampsia had a gestational age of 36 - 37 weeks. Preeclampsia is divided into two subtypes: early (before 34 weeks of gestation) and late - onset (after 37 weeks). There is also a classification that includes the intermediate onset

subtype (34 - 37 weeks). One of the main differences between these subtypes is fetal growth restriction in early - onset preeclampsia.⁸ Another difference is the presence of placental pathology in the form of uteroplacental mal - perfusion in early - onset preeclampsia. It is associated with failure of the spiral arteries to change in early pregnancy (8 - 18 weeks, poor placentation) leading to a too - small diameter and poor contractility. This causes the spiral arteries to be unable to facilitate the non - pulsatile, large - volume, and low - pressure blood flow required by the placenta in the third trimester. As the result, oxidative stress increases which can cause damage to the infarction of the placental tissue. Spiral arteries can also be obstructed by acute atherosclerosis and atherosclerosis - like lesions that can lead to arterial thrombosis. In late - onset preeclampsia, there is evidence suggesting that there is diffuse placental hypoxia due to placental growth exceeding the capacity of the uterus and its vasculature to meet the demands of the placenta at term. Thus, early and late - onset preeclampsia has the same etiology: mal - perfusion, although they have different causes.

Based on the APGAR assessment, 21 neonates (70%) showed an APGAR score of 7/8. The average birth weight is 2920 grams with the lowest weight being 2500 grams and the highest weight being 4000 grams. In contrast to the study conducted by Mamo SA, et al, the results showed that there was a significant relationship between preeclampsia and the incidence of asphyxia neonatorum with APGAR < 7, the result of the analysis was p - value 0.007.9 Mothers diagnosed with preeclampsia were 2.9 times more likely to give birth to babies with neonatal asphyxia than mothers who were not diagnosed with preeclampsia.⁹

Preeclampsia causes reduced blood flow to the uterus which causes reduced oxygen flow to the placenta and fetus. Vasoconstriction of blood vessels results in reduced blood supply to the placenta, resulting in fetal hypoxia. A further consequence of fetal hypoxia is impaired gas exchange between oxygen and carbon dioxide, resulting in neonatal asphyxia. Neonatal asphyxia is characterized by the APGAR score of neonates < 7. Factors that cause the occurrence of asphyxia neonatorum are antepartum factors including parity, maternal age, hypertension, hemoglobin levels, and antepartum bleeding. Intrapartum factors include presentation, duration of labor, state of amniotic fluid, and type of delivery. As well as fetal factors: prematurity and birth weight. The risk factor discussed in this study is preeclampsia.¹⁰

Based on the baby's birth weight, our study showed that most of the babies from the pregnancy group with preeclampsia were born weighing 2500 - 4000 grams. This is because our research subjects fall into the category of late - onset preeclampsia. However, the number of babies born weighing less than 2500 grams was more than in the normal pregnancy group. Getaneh et al, 2020 stated that preeclampsia can increase the risk of low birth weight compared to the control group.¹¹

The results of the analysis using the dependent T - test showed that there was no significant difference in the mean NLR before and after the termination of pregnancy (p =

0.091). The mean and median NLR values were slightly higher in the preeclampsia group before termination than in the preeclampsia group after termination. There are different results regarding the value of NLR in preeclampsia before termination and after termination in previous studies. Zheng et al, 2019¹² compared the NLR in preeclampsia patients before termination compared to the preeclampsia group after termination, but found no significant difference. However, in a study by Singgih et al, 2021, NLR was found to be significantly increased in the preeclampsia group before termination when compared to the preeclampsia group after termination. The NLR value was also significantly higher in preeclampsia patients with aggravating symptoms than in patients without aggravating symptoms, indicating an association between the severity of preeclampsia and the NLR value.¹³

In preeclampsia, there is inadequate placentation, due to a deficiency of trophoblast invasion which will cause placental hypoxia, secretion of proinflammatory cytokines, and release of angiogenic and antiangiogenic factors. Changes in the immune system are one of the things studied as a cause of preeclampsia, especially the role of proinflammatory cytokines, neutrophil activation, and endothelial dysfunction. Pregnancy is a controlled inflammatory state. There is a theory that an exaggerated systemic inflammatory response is the basis for the systemic manifestations of preeclampsia, although the exact mechanism remains unclear. All components of maternal leukocytes such as neutrophils, lymphocytes, and monocytes are activated during pregnancy, with higher levels in preeclamptic patients. This activation is thought to be associated with the vascular dysfunction that occurs in preeclampsia.¹³

Neutrophils are the body's first line of defense against infection in the event of an injury. There are studies that suggest that neutrophils also infiltrate the systemic vascular tissue in women with preeclampsia causing systemic vascular inflammation. In women with preeclampsia, neutrophils are activated as they circulate through the intervillous space and are exposed to oxidized lipids secreted by the placenta. Neutrophils from women with preeclampsia expressed more cyclooxygenase - 2 than neutrophils from women with pregnancies without preeclampsia or women who were not pregnant. However, the mechanism behind this immune system modulation has not been elucidated. Neutrophils are often considered the body's first line of defense against infection at the wound site. Recent studies have reported that neutrophils infiltrate the systemic vascular tissue in women with preeclampsia, causing vascular inflammation. All classes of leukocytes are activated in the maternal circulation of women with preeclampsia, but only neutrophils significantly infiltrate the systemic vasculature. The number of neutrophils in blood vessels is three times more than that of lymphocytes. There are also research results that state that there is a 2.5 - fold increase in the number of neutrophils at gestational age above 30 weeks and a higher increase in patients with preeclampsia.¹³

In conclusion, in this study, there was no significant difference in the mean NLR before and after the termination

of pregnancy. Further studies are needed involving a larger number of subjects, dividing subjects with early or late onset preeclampsia and associated with other inflammatory markers such as CRP or with coagulation status such as D-dimer.

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